E. COLI DERIVED SPIDER SILK MASP 1 AND MASP 2 PROTEINS AS CARBON FIBER PRECURSORS

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Utah State University

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Project ID LM103

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Overview

Timeline

Start date: November 1, 2014

End date: March 31, 2017

Percent complete: 90+%

Barriers

- 2.5.1. Lightweight Materials Technology (VTP MYPP 2011-2015)
  - Performance: Match carbon fiber using spider silk instead of PAN

Budget

- Total project funding
  - DOE: $1,490,744
  - Contractor share: $497,298
- Funding FY 2015: $997,758
- Funding FY 2016: $990,284

Partners

- U. of California, Riverside
- Oak Ridge Nat'l Laboratory
- Utah State University

Relevance

Overall Project Objective

Reduce the weight of vehicles thereby reducing green house gas emissions and the dependence on foreign oil through the use of carbon fibers produced from spider silk protein fibers

Project Goals

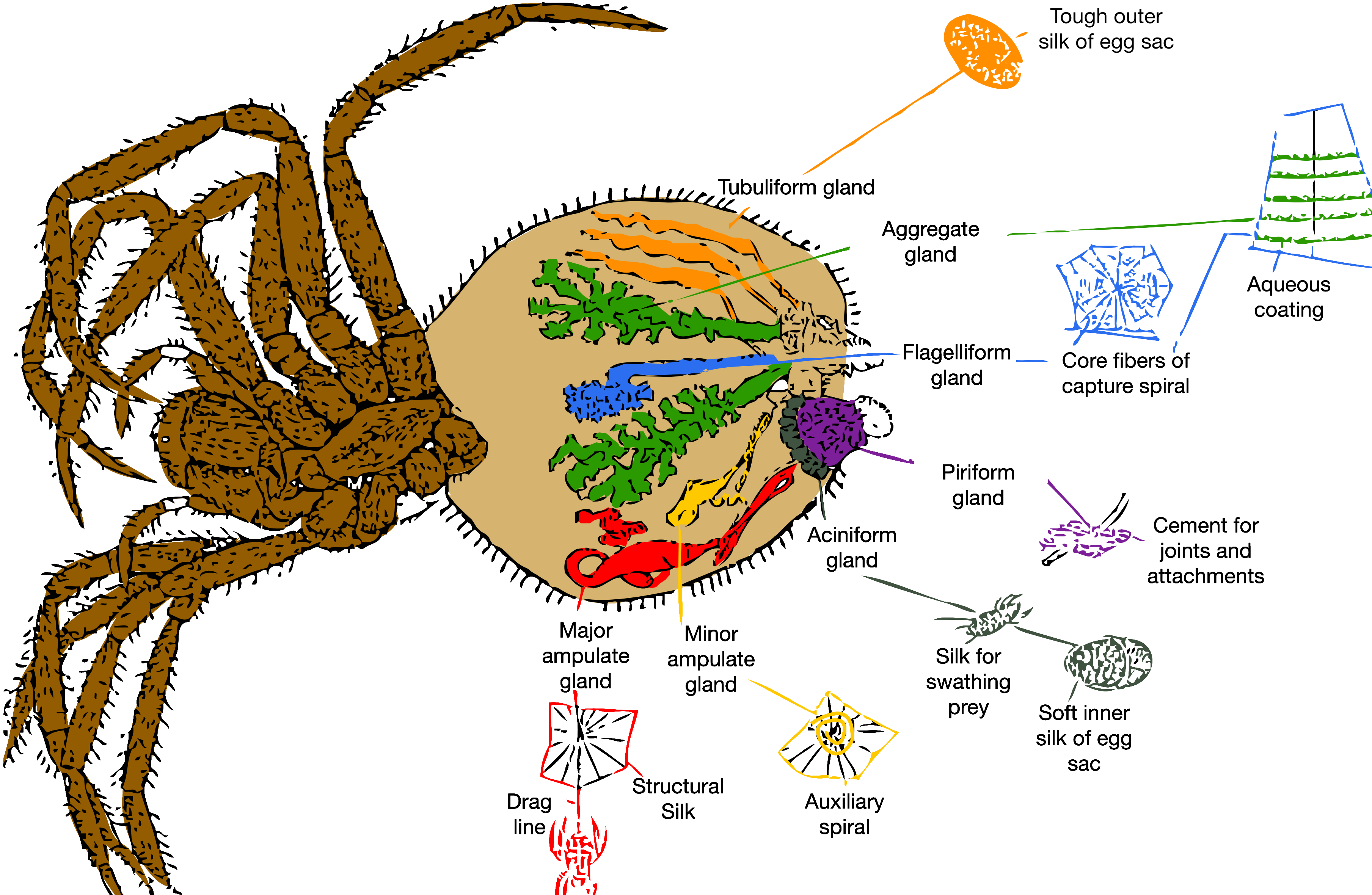
- Maximize protein production via *E. coli* while maintaining full-length protein
- Develop a Scalable Fiber Spinning process
- Improve spider silk fiber mechanical properties
- Generate transgenic silkworms producing silk with much higher strength
- Determine optimal stabilization conditions for spider silk protein fibers for conversion to carbon fibers
- Conduct techno-economic analyses to estimate costs

Milestones

Recipient Name: Randolph V. Lewis, Utah State University							
Project Title: Spider Silk MaSp1 and MaSp2 Proteins as Carbon Fiber Precursors							
Task #	Task Title	Milestone type	Milestone number	Milestone description	Milestone verification	Percent Completion	Expected Quarter
1	Fiber production	Milestone	1.1.1	1g/L protein	Purified protein recovered	100	Q2
1	Fiber spinning	Milestone	1.2.1	Tensile strength	Mechanical testing	100	Q3
1	Silkworm transgenesis	Milestone	1.3.1	Silk tensile strength	Mechanical testing	100	Q3
1	Spider silk production	Go/No		Tensile strength	Mechanical testing	100	Q4
2	Conversion	Milestone	2.1.1	Pre-treatment	Carbonization	100	Q5
2	Conversion	Milestone	2.2.1	Carbon fiber strength	Mechanical testing	100	Q6
2	Conversion	Go/No		Stabilized fiber	Thermal stability	100	Q7
2	Conversion	Milestone	2.3.1	Strength	Mechanical testing	90	Q8
2	Conversion	Milestone	2.4.1	Property relationships	Micro-structure	75	Q7
3	Technoecon	Milestone	3.1.1	Validation of sub models	Experimental Verification	100	Q1
3	Technoecon	Milestone	3.1.2	Engineering system model	Sensitivity Analysis	100	Q2
3	Technoecon	Milestone	3.2.1	TEA	Technology Comparison	100	Q3
3	Technoecon	Milestone	3.2.2	Process Optimization	Economic Viability	100	Q4
3	Technoecon	Milestone	3.3.1	LCA	Technology Comparison	100	Q6
3	Technoecon	Milestone	3.3.2	Vehicle Modeling	Impact Comparison	100	Q8

Approach/Strategy

- Create spider silk fibers with tensile strength of >750 MPa (Go/No Go with intermediate milestones) **Achieved Q4**
- Convert spider silk fibers to stabilized carbon fibers (Go/No GO, Q7 with intermediate milestones) **Achieved Q6**
- Techno-economic analysis of estimated production costs (Final milestone Q8, with intermediate milestones) **Achieved Q8**



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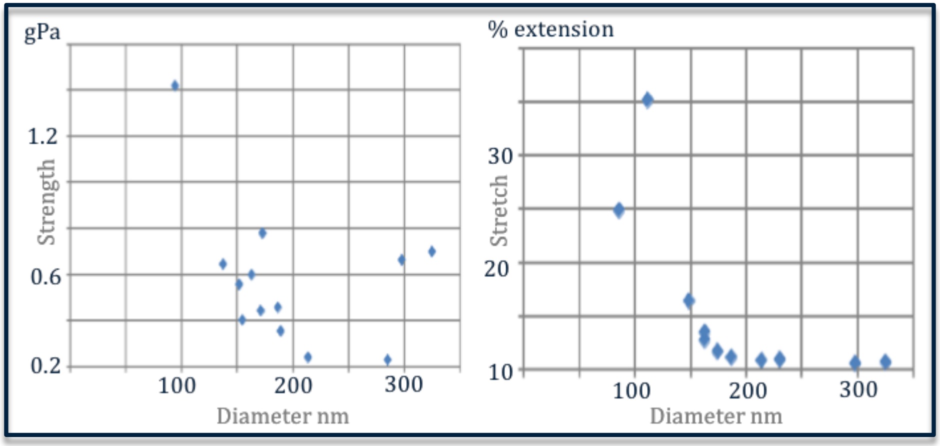
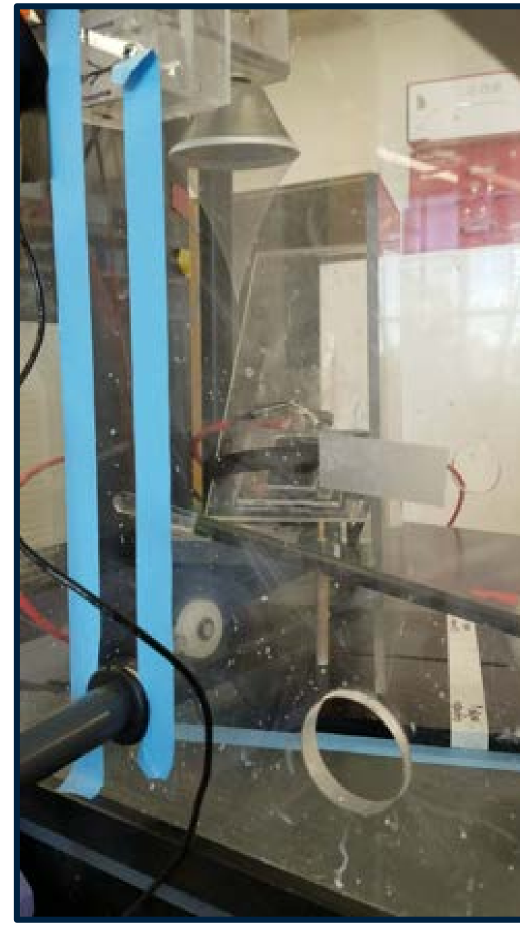
Technical Accomplishments and Progress

- Create spider silk fibers with tensile strength of >750 Mpa

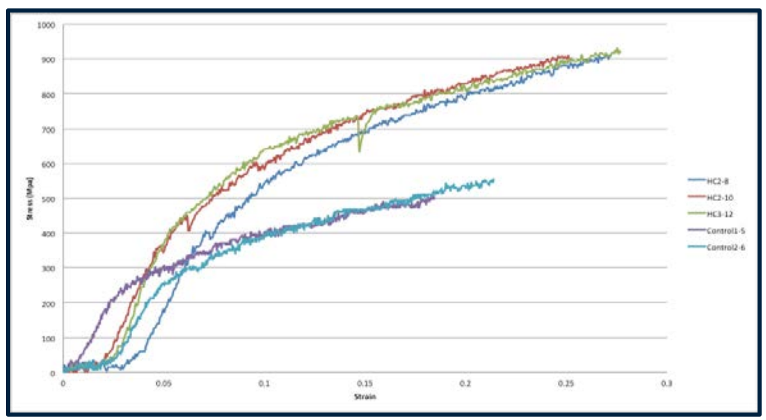


Electrospun spider silk protein fibers ranging from 100-350nm with corresponding tensile strengths and elongations. Note the non-linear behavior of both properties.

Continuous electro-spinning to produce nanoscale fibers in a filament.



Spools of bacterially produced spider silk protein, 350m of 8-fiber thread.



Stress-strain curves for control and transgenic silkworm silk. The samples are the same as described above in the table above. Note both the similar shapes and values for the different transgenic silkworm lines which is very similar to the variation in the controls.

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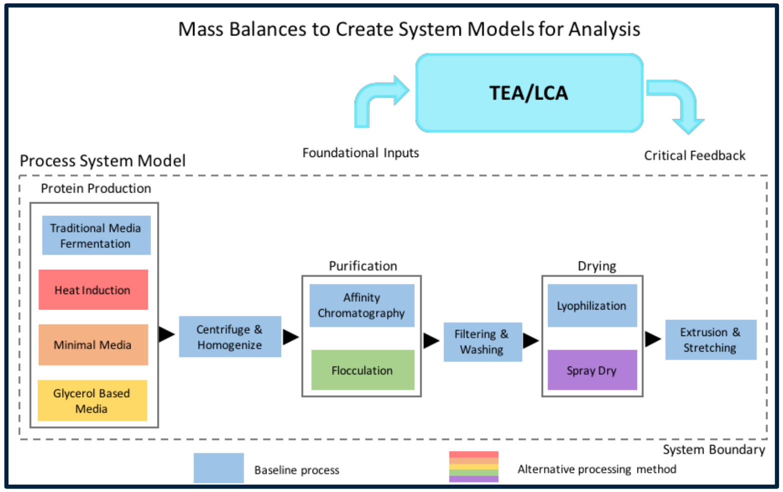
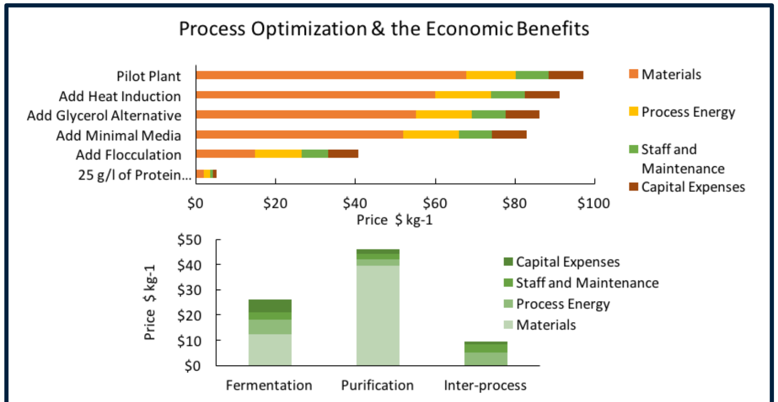
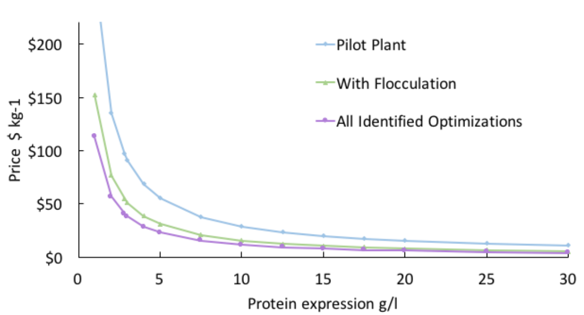
Technical Accomplishments and Progress

- Techno-economic analysis of estimated production costs





- Costs are driven most significantly by the material consumption, specifically by purification
- Research is being focused towards reducing material consumption
- Process optimization can significantly reduce the cost of production



- Protein yield has the most direct impact on sale price
- Increasing yield has little effect on cost, but significantly reduces the sale price estimate
- Other high yielding recombinant operations have yields above 20 g/l

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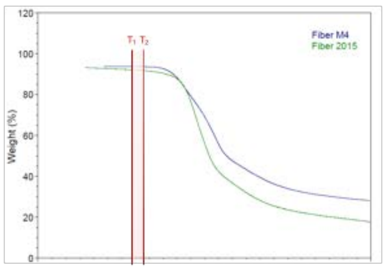
Technical Accomplishments and Progress

- Convert spider silk fibers to stabilized carbon fibers

Stretch Study of Fiber 2015 and Fiber M4 (Jan 2016)



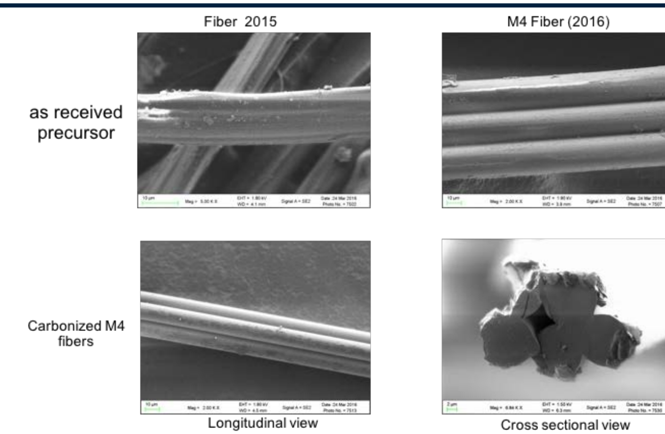
Thermogravimetric curves of Fiber 2015 and Fiber M4 (2016) in N_2:

Produced carbon fibers from M4 precursor can be easily wrapped around a core with a diameter of 1"

- M4 showed higher yield than previous fiber
- Stretching window has been identified between T_1 and T_2 for both materials at the beginning of the process
- New precursor Fiber M4 has shown better stretch characteristics during process. (this potentially leads to higher mechanical performance)
  - Fiber 2015 : Max 4.6%
  - Fiber M4 (2016) : Max 25.6%

→ M4 Fibers can be stretched typically up to 20% during process (carbonized batched obtained on Apr 5th, 2016)

Characterization of the samples: SEM



- Samples at all temperature looked similar (1700°C is shown)
- Original morphology of the filament well preserved, despite:
  - Exposition to high temperature (up to 1700°C)
  - 75% weight loss (mostly below 1000°C)
  - 3 thermal treatments (usage of 3 furnaces)

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III. Characterization of the samples: Mechanical properties

- First time filaments of spider silk are successfully carbonized
- All tested specimens are bundles of 8 fused filaments (due to initial morphology of the precursor) with an average of 12 measurements per test

Sample ID	Max. temp. of treatment [°C]	Equivalent diameter [µm]	peak stress [ksi]	Modulus [Msi]	Strain at break [%]
Bundles type 1	1300	29.37 (1.58)	99.7 (41.0)	7.5 (0.9)	1.28 (0.42)
Bundles type 2	1300	29.34 (1.51)	77.3 (41.1)	7.3 (1.1)	1.06 (0.67)
Bundles type 3	1500	28.52 (1.35)	69.2 (43.6)	8.9 (1.4)	0.78 (0.50)
Bundles type 4	1700	26.08 (3.11)	101.9 (61.9)	7.4 (2.0)	1.32 (0.67)

100ksi (30% higher than aerospace carbon fiber) achieved on batch process without control of the tension above 1000°C

→ 2 immediate paths for improvement:

- Precursor: unfused filaments
- Optimization of the process of conversion (tension at high temperature)

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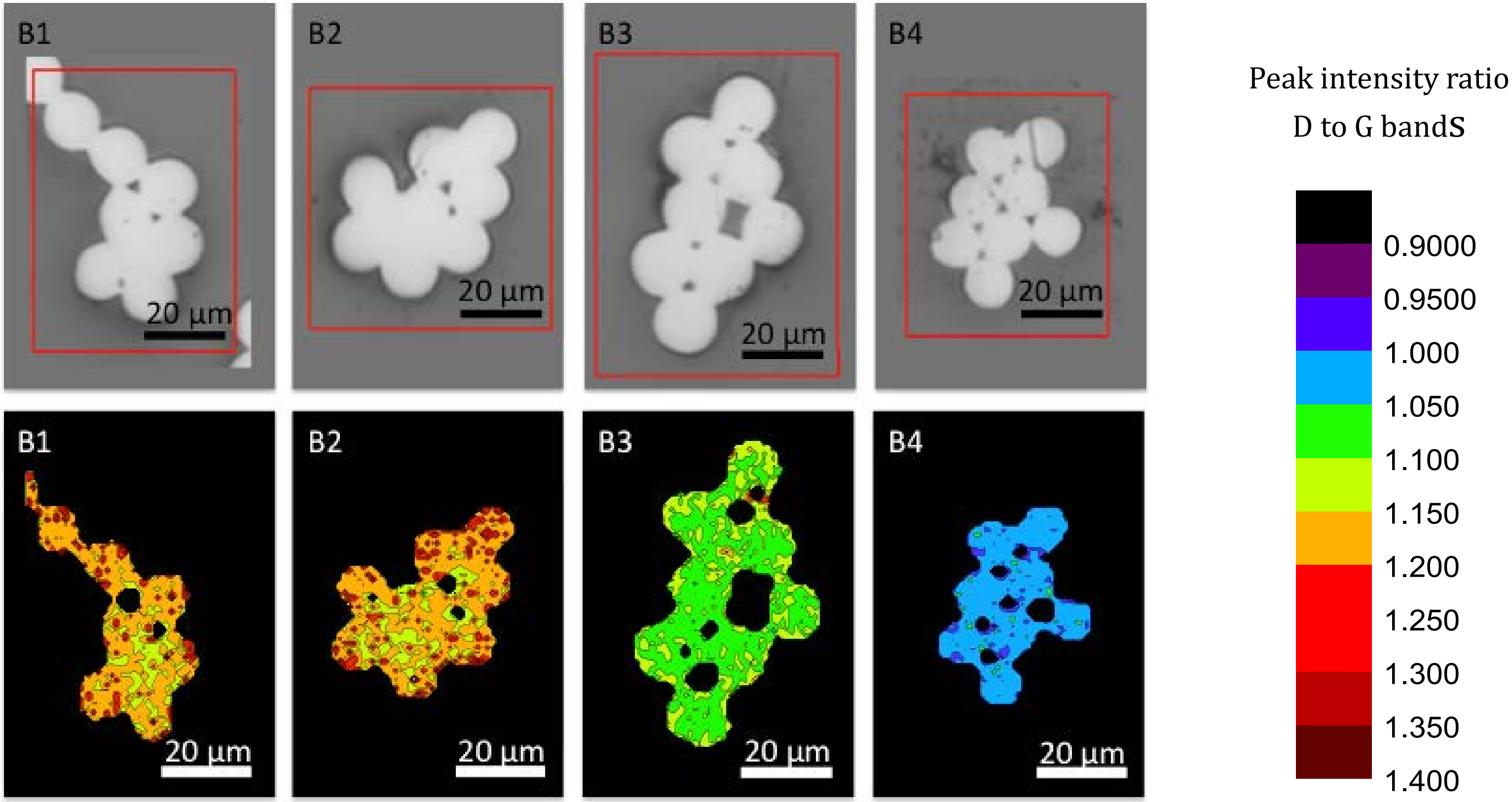
III. Characterization of the samples: Raman spectra (cross-section)



Optical images (top row) and maps (bottom row) of the Raman mapping of peak intensity of D to G on cross section of CF bundles (B1 to B4, 1300, 1300, 1500, 1700°C respectively).

The value of the ratio (I_D/I_G) is represented by a color, G peak corresponds to *sp2* bonds and the D peak corresponds to *sp3* bonds → Defects [Low ratio (I_D/I_G) → low defects]

Elevation of temperature → reduce defects and increase homogeneity

D/G decreases moving across the series from B1 to B4.

Best sample: B4 (1700°C) more homogeneous with less defects

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Response to Previous Year Reviewer's Comments

The major concern was the conversion process which as shown above was successful and generated fibers with excellent tensile strength.

Partners and Collaborators

- Dr. Cheryl Hayashi, U. of California, Riverside, co-PI.
  Gene sequences and comparisons for spider silk protein gene choices to produce.
- Drs. Soydan Ozcan and Felix L.Paulauskas, ORNL co-PIs.
  Spider silk fiber conversion to carbon fiber and analyses of those fibers.
- Dr. Jeff Yarger, Arizona State University, collaborator.
  NMR, Raman and X-ray diffraction.
- Argonne National Laboratory, facilities.
  X-ray diffraction facility

Remaining Challenges and Barriers

- Generate better spider silk fibers for stronger carbon fiber
- Increase spider silk protein production to drive costs down
- Better conversion:
  - work on continuous and thicker tows
  - reduce the time of conversion
  - increase the char yield (for a better production rate)

Proposed Future Work

No funding available to continue the project but if there were:

Fibers:

- Make unfused multi-fiber filaments
- Increase percentage of ß-sheets for better CF fibers
- Increase protein production to continue to drop costs

Paths for future improvement of conversion:

- Work on unfused filaments
- Test new spider silk fibers
- Improve the crystallization by having a better control of the tension during the all process, and especially above 1000^{o}C

Any proposed future work is subject to change based on funding levels.

Summary

- Maximized protein production via *E. coli* while maintaining full-length protein
  - Protein production has gone from 0.5g/L to as high as 4.0 g/L
  - Purification process developed with 17-fold lower costs

- Developed a Scalable Fiber Spinning process
  - Up to 1000m of 8 fiber thread has been spun
  - Moving to a 24 fiber thread spinning head

- Improve spider silk fiber mechanical properties
  - Improved from 200 MPa to over 400 Mpa

- Spider silk protein fibers converted to carbon fibers
  - Tensile strength 30% higher than aerograde carbon fiber
  - Excellent homogeneity at 1500°C and above
  - Morphology of bundles preserved
  - Fibers easily manipulated

- Techno-economic analyses estimate optimal costs as low as $5-10/kg

Technical Back-Up Slides

Special Mechanical Properties of Spider Silks

Material	Strength (MPa)	Strain (%)	Toughness (KJ/kg)
Dragline silk	4000	35	400
Minor Ampullate silk	1000	5	30
Flagelliform	1000	>200	400
Tubiliform silk	1000	20	100
Bombyx mori silk	600	20	60

[a]Data from Gosline,Lewis, Altman

Production Methods

System	Protein Yield per Year	Production Time
Bacteria	12 kg per run	2-4 months
Goats	18 kg per goat	1-2 years
Alfalfa	218 kg per acre	4-5 years
Silkworm	??	2 years